Mechanisms of Stimulation of Granulocytopoiesis with Neupogen in Patients with Breast Cancer during Chemotherapy

T. Yu. Khrichkova, V. E. Gol'dberg, V. V. Zhdanov, M. G. Matyash, V. V. Vysotckaya, E. I. Simolina, V. A. Shatalova, N. O. Popova, L. A. Miroshnichenko, and A. M. Dygai

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The mechanisms of granulocytopoiesis stimulation with granulocytic CSF (neupogen), added to chemotherapy protocol (adriablastine+taxotere) in patients with stages III-IV breast cancer, were studied. The hemostimulatory effect of granulocytic CSF preparation is based on stimulation of proliferation and differentiation of granulomonocytopoisis precursor cell in the hemopoietic tissue, due to neupogen effects on hemopoietic elements and on the hemopoiesis-inducing microenvironment cells.

Key Words: *granulocytic colony-stimulating factor; hemopoietic precursors; granulocytopoiesis; chemotherapy*

Numerous clinical studies demonstrated high efficiency of taxotere in combination with antracyclines in the treatment of disseminated breast cancer (BC), due to which some authors regard this combination as a new standard of chemotherapy for disseminated BC forms [4,11].

A frequent complication of taxotere and adriablastine therapy is suppression of bone marrow hemopoiesis, largely of the granulocytic stem, which manifests by development of leukopenia with predominant reduction of the level of neutrophilic granulocytes in the peripheral blood. More rare side effects are anemia and thrombocytopenia [4]. Analysis of the data confirmed the development of the III-IV degree neutropenia in 85-100% patients and of febrile neutropenia in 30-40% patients receiving cytostatic therapy according to this protocol [7]. The obvious clinical significance of myelosuppression consequences often justifies and necessitates prescription of neupogen as a hemostimulant [8,12].

We studied the efficiency of neupogen as a corrector of disorders in the granulocytic stem in patients with stages III-IV BC during antitumor chemotherapy according to the adriablastine+ taxotere protocol and the mechanisms underlying the hemostimulatory effect of this drug.

MATERIALS AND METHODS

The data obtained in studies of the blood system of 77 patients (aged 35-55 years) with stages III-IV BC were used in the study.

All patients received antitumor polychemotherapy according to the following protocol: 50 mg/m^2 adriablastine (Pharmacia and Upjohn S.p.A.) on day 1 and 75 mg/m^2 taxotere (Aventis Pharma, S.A.) on day 2. The cycle duration was 2 days, with 3-week interval between the courses. Blood system values were studied during 3 chemotherapy courses. In addition to the above treatment, 30 patients recei-

Institute of Pharmacology and Institute of Oncology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences, Russia

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ved neupogen between the courses (300 μg subcutaneously on days 8 and 12 after the last injection of taxotere) [12]. Control group consisted of patients receiving no neupogen.

Material for the study (capillary and venous blood) was collected before and after each cycle of cytostatic therapy. Sternal punctures were carried out before special treatment and before chemotherapy courses 2 and 3.

Peripheral blood tests and differential evaluation of myelograms in sternal puncture specimens were carried out by the standard hematological methods [3]. Colony-forming capacity of the bone marrow and peripheral blood was evaluated by cloning granulomonocytopoiesis precursor cells (CFU-GM) in semiviscous methyl cellulose-based nutrient medium [2]. The intensity of hemopoietic precursor differentiation was evaluated by the maturation index. Proliferative activity of CFU-GM was studied using hydroxyurea [2]. The levels of colony-stimulating activity (CSA) in media conditioned by adherent and nonadherent elements of the hemopoiesis-inducing microenvironment and in the sera were measured in semisolid medium on intact mouse myelokaryocytes [2].

The data were processed by methods of variation statistics using Student's *t* test.

The protocol of the study was approved by the Ethic Committee of Institute of Oncology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences.

RESULTS

Total leukocyte count in the studied patients decreased by 49.1% from the initial level after chemotherapeutic course 1 (Fig. 1, *a*). The resultant leukopenia was caused mainly by reduction of the absolute count of segmented neutrophils (Fig. 1, *b*). By course 2, the total leukocyte count in patients trea-

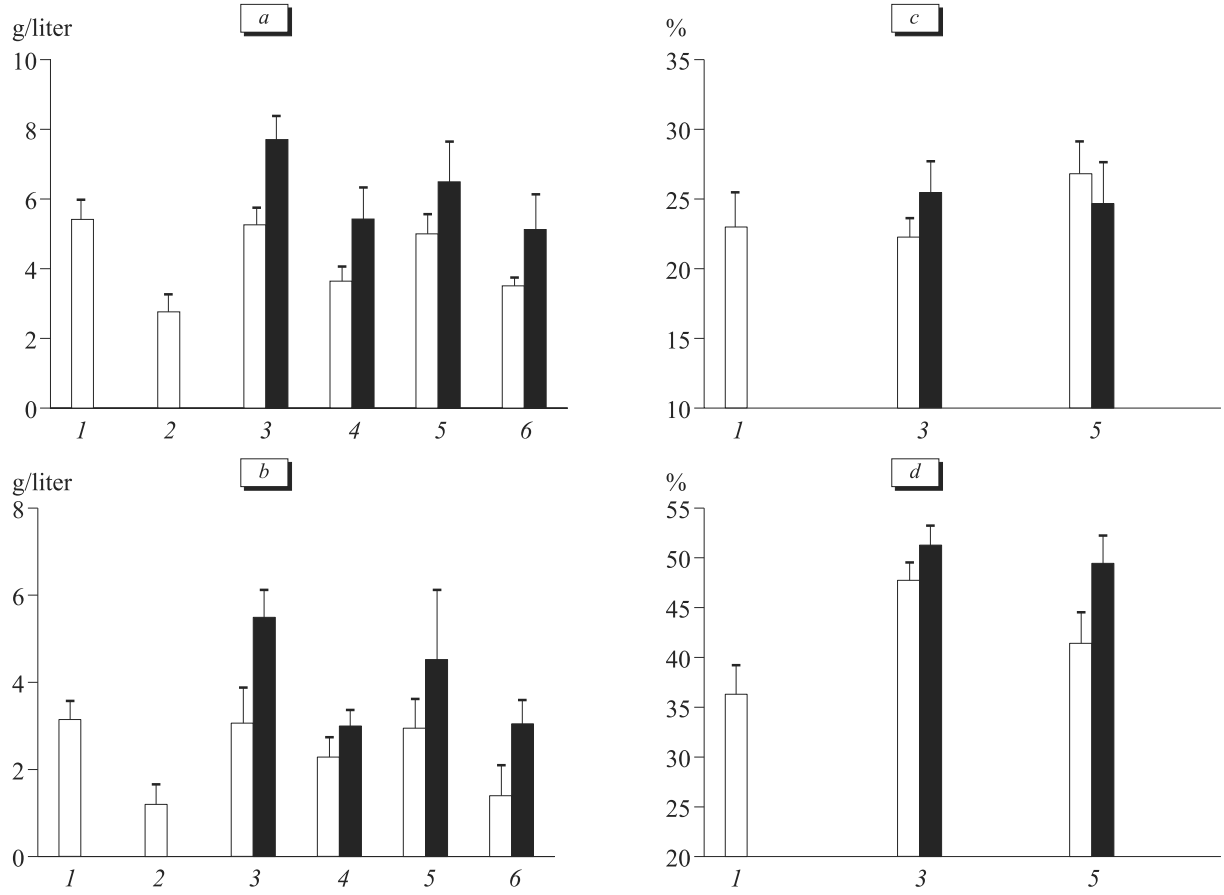

Fig. 1. Dynamics of total leukocyte count (*a*), counts of segmented neutrophils (*b*) in peripheral blood, immature (*c*) and mature (*d*) neutrophilic granulocytes in the bone marrow of patients with stages III-IV BC during antitumor chemotherapy with adriablastine and taxotere (light bars) or their combination with neupogen (dark bars). Abscissa: period of the study: *1*) before therapy; *2*) after course 1; *3*) before course 2; *4*) after course 2; *5*) before course 3; *6*) after course 3. Confidence intervals at $p<0.05$.

ted with neupogen was significantly higher than their initial level and the values in the control group due to the increase in the count of segmented neutrophilic granulocytes in the peripheral blood (Fig. 1, *a*, *b*).

A significantly higher level of leukocytes in the peripheral blood of patients treated with neupogen was recorded after chemotherapeutic course 2; this was due to not only segmented neutrophils, but

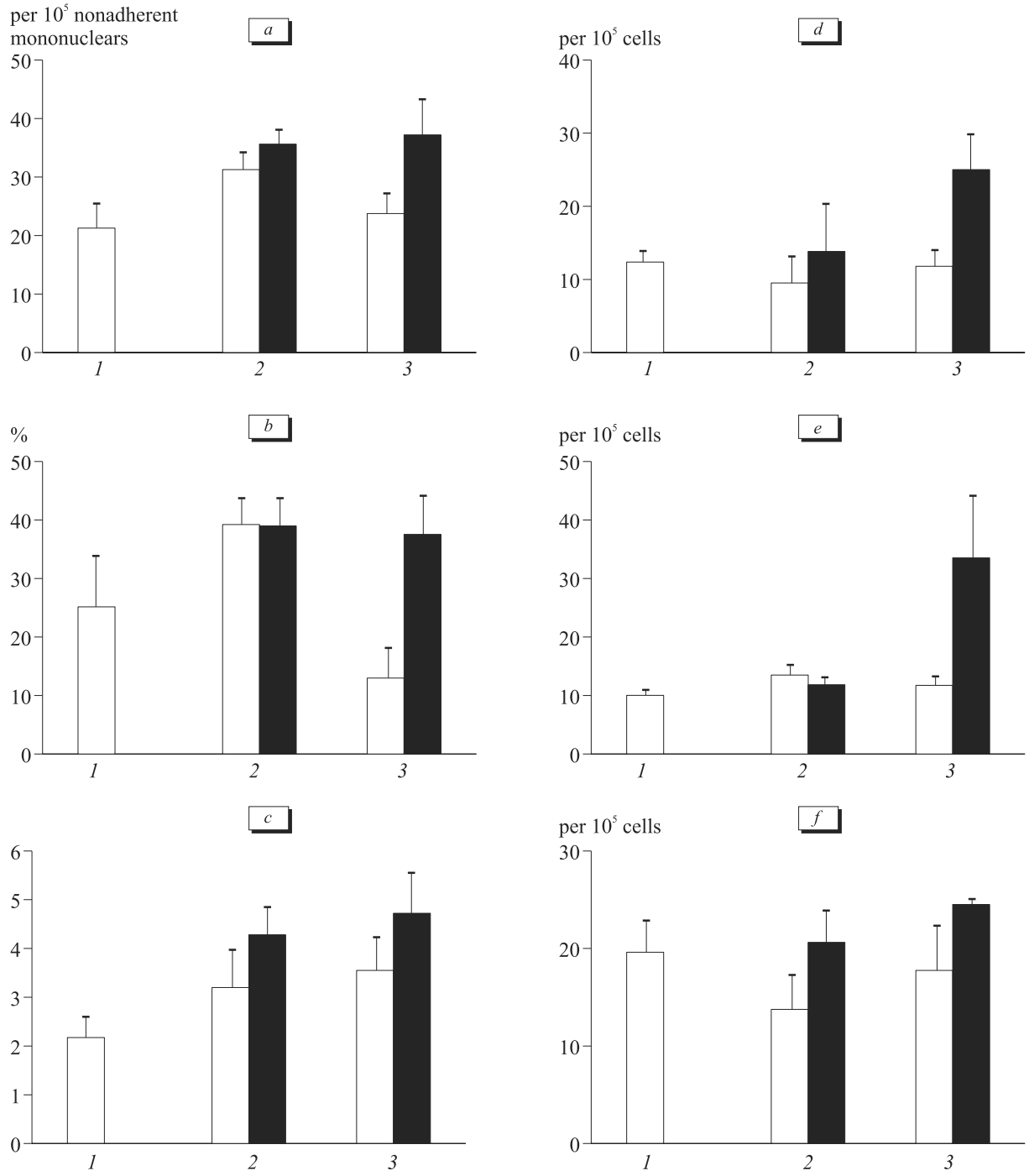

Fig. 2. Dynamics of CFU-GM content (*a*), percent of S-phase CFU-GM (*b*), intensity of CFU-GM maturation (*c*) in the bone marrow, and CSA in supernatants from adherent (*d*), nonadherent myelokaryocytes (*e*) and in the sera (*f*) of patients with stages III-IV BC during antitumor chemotherapy with adriablastine and taxotere (light bars) or their combination with neupogen (dark bars). Abscissa: period of the study: *1*) before therapy; *2*) before course 2; *3*) before course 3. Confidence intervals at $p<0.05$.

TABLE 1. Peripheral Blood Count of CFU-GM (per 10^5 Nonadherent Mononuclears) in Patients with Stages III-IV BC during Adriablastine+Taxotere (*A*) or Adriablastine+Taxotere+Neupogen Chemotherapy (*B*; $X \pm m$)

Course						
1		2			3	
before	after		before	after	before	after
27.18±2.72	23.52±1.85	A	24.92±2.04	23.04±2.73	26.12±2.04	25.88±2.21
		B	33.11±1.67$^+$	36.38±2.62^{*+}	31.45±1.23$^+$	31.38±1.12$^+$

Note. $p<0.05$ compared to: *values before therapy, $^+$another group.

also lymphocytes and monocytes. Later, after the 2nd course of corrector drug, the levels of total leukocytes and their segmented forms in the peripheral blood of patients injected with hemopoietin were significantly higher in comparison with the control (Fig. 1, *a*, *b*).

Analysis of sternal puncture specimens showed increased (14.3%, $p<0.05$, compared to the control group) content of immature neutrophilic granulocytes (myeloblasts, promyelocytes, myelocytes, metamyelocytes) in hemopoietic tissue by chemotherapy course 2 in patients treated with neupogen (Fig. 1, *c*). The counts of mature neutrophilic granulocytes (stab and segmented neutrophils) increased significantly before courses 2 and 3 of special treatment (by 7.4% and 19.4%, respectively, Fig. 1, *d*). Presumably, these changes were due to the absence of appreciable damage to granulocytopoiesis precursors capable of mitosis inflicted by adriablastine combination with taxotere, this creating prerequisites for activation of regenerative processes in the bone marrow under the effect of hemostimulant at the early stages of the cytostatic disease [1] and indirectly indicates increasing activation of proliferation and differentiation of granulocytic cells during chemotherapy supplemented with neupogen.

Evaluation of the bone marrow colony-forming activity confirmed these hypotheses. CFU-GM count increased by 14 and 56.6%, respectively, by the start of chemotherapy courses 2 and 3 supplemented with neupogen in comparison with the control, which can be due to active proliferation of hemopoietic precursor under the effect of the cytokine (Fig. 2, *a*). The count of DNA-producing CFU-GM in the hemopoietic tissue of BC patients increased 1.5 times during chemotherapy combined with neupogen in comparison with the initial level ($p<0.05$); before course 3 of special therapy (including corrector drug) this parameter 2.9 times surpassed the control value (Fig. 2, *b*; $p<0.05$). Therapy by the common protocol led to a significant decrease (1.94 times; $p<0.05$) in proliferative activity in comparison with the initial level by the start of course 3 of cytostatic therapy (Fig. 2, *b*). It is known that quantitative status of the pool of any immature cells is determined by not only proliferation intensity, but also the rate of differentiation [1]. Additional estimation of the number of clusters in hemopoietic cell cultures provided data demonstrating a significant ($p<0.05$) increase in granulocytic precursor maturation index during chemotherapy supplemented with neupogen in comparison with the basal level (2.1 times) and with the control (1.33 times; Fig. 2, *c*).

Hence, changes in the pool of committed precursors play an important role in the stimulation of postcytostatic granulomonocytopoiesis regeneration under the effect of granulocytic CSF preparation (G-CSF). Our data are in line with previous findings indicating that the hemostimulatory effect of G-CSF is due to activation of proliferation and differentiation of the respective precursors [9,10].

Neupogen-stimulated increase in the number of granulocytomacrophage precursors in the bone marrow was paralleled by an increase in their counts in the peripheral blood manifesting in a significant accumulation of CFU-GM at all terms of the study in comparison with the control; the peak number of CFU-GM (1.6 times) was observed after course 2 (Table 1). These data can be explained, in addition to increased proliferative activity of committed precursors in the bone marrow, by neupogen-stimulated redistribution reactions in the blood system, because the detected changes correspond to the picture of hemopoietic precursor mobilization from the bone marrow to peripheral blood. The mobilizing effect of G-CSF preparation is widely used in clinical practice for collection of $CD34^+$ cells for subsequent autologous transplantation [6].

Cells of the hemopoiesis-inducing microenvironment modify proliferation and differentiation of hemopoietic precursors via production of a wide spectrum of short-distance humoral factors [1]. Therefore, for further studies of the mechanisms under-

lying the stimulatory effect of G-CSF preparation on bone marrow hemopoiesis in BC patients during chemotherapy we determined CSA levels in supernatants of bone marrow nuclears. A sharp increase in secretory activity of adherent and nonadherent bone marrow cells was noted before course 3 of chemotherapy with neupogen. The use of hemopoietin at this stage led to a significant increase of CSA in comparison with the control group: 2.1 times for bone marrow adherent cells and 1.6 times for nonadherent cells (Fig. 2, *d*, *e*). The involvement of secretory products of the microenvironment cell into activation of hemopoiesis under the effect of G-CSF indicates indirect route of realization of the hemostimulatory activity of the preparation.

The increase in serum CSA level in patients receiving neupogen in addition to the cytostatic protocol 1.5 times in comparison with the group receiving no corrector drug (Fig. 2, *f*; $p<0.05$) attests to a systemic stimulatory effect of neupogen on secretory activity of accessory cells. CSA is an integrative index characterizing the summary effect of bioactive substances present in the serum. Since G-CSF half-life period after subcutaneous injection is 3-4 h, by the moment of our test, exogenous CSF was eliminated and hence, its contribution to serum CSA is negligible [5].

Hence, addition of neupogen to adriablastine+ taxotere chemotherapy leads to an increase in the total leukocyte count, suppressed with the cytostatics, largely due to the increase in the count of their segmented forms. These changes are caused by accumulation of morphologically differentiated cells and committed granulocytopoiesis precursors in the bone marrow due to activation of their proliferation and differentiation. These effects of neupogen can be explained by direct effects on hemopoietic cells and by modification of functional activity of the hemopoietic microenvironment cells. The production of hemopoietic growth factors by microenvironment cells significantly increased. In addition, neupogen treatment stimulated the release of hemopoietic precursors of different classes into peripheral blood and increases serum CSA level.

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